

## Claims

We claim:

1. A method for synthesizing a nucleic acid molecule comprising at least one non-canonical nucleotide, comprising the steps of:

5 a) incubating a template nucleic acid in a reaction mixture under nucleic acid synthesis conditions containing (i) a mutant nucleic acid polymerase, wherein said polymerase has a reduced discrimination between canonical and non-canonical nucleoside triphosphates, and (ii) at least one non-  
10 canonical nucleoside triphosphate, wherein said non-canonical nucleoside triphosphate is incorporated into the synthesized nucleic acid in place of only one canonical nucleoside triphosphate, and

15 b) obtaining the synthesis of a nucleic acid molecule comprising at least one non-canonical nucleotide.

2. The method of claim 1 wherein the template nucleic acid is DNA.

3. The method of claim 1 wherein the template nucleic acid is RNA.

4. The method of claim 1 wherein a nucleic acid molecule comprising at least one non-canonical nucleotide is synthesized by extension of a primer molecule, at least part of which is sufficiently complementary to a portion of the  
5 template to hybridize therewith.

5. The method of claim 1 wherein a nucleic acid molecule comprising at least one non-canonical nucleotide is synthesized *de novo* without using a primer molecule.

6. The method of claim 1 wherein the polymerase is an RNA polymerase.

7. The method of claim 1 wherein the polymerase is a T7-type RNA polymerase.

8. The method of claim 1 wherein the polymerase is selected from the group consisting of T7 and SP6 RNA polymerases.

9. The method of claim 1 wherein the mutant polymerase is an RNA polymerase and the non-canonical nucleoside triphosphate is a 2'-fluoro-nucleoside triphosphate.

10. The method of claim 1 wherein the synthesized nucleic acid molecule has an altered susceptibility to a ribonuclease or a deoxyribonuclease compared to a nucleic acid which is synthesized using the corresponding non-mutant  
5 nucleic acid polymerase.

11. The method of claim 1 wherein the synthesized nucleic acid molecule is selected from the group consisting of a ribozyme or a nucleic acid molecule used for gene therapy, in a vaccine, in an antiviral composition, in an antimicrobial composition, in an antisense composition for regulating gene expression, in a composition for hybridization to a complementary nucleic acid, or as a probe for detection of a complementary nucleic acid.

12. The method of claim 1 wherein the synthesized nucleic acid molecule is single-stranded.

13. A kit for performing the method of claim 1, comprising a mutant nucleic acid polymerase which has reduced discrimination between canonical and non-canonical nucleoside triphosphates and data or information describing conditions under which the method of claim 1 may be performed.

14. The kit of claim 13, wherein the nucleic acid polymerase is a mutant T7-type RNA polymerase.

15. The kit of claim 13, wherein the nucleic acid polymerase is a T7 RNA polymerase comprising an altered amino acid at position 639.

16. The kit of claim 13, wherein the nucleic acid polymerase is SP6 RNA polymerase comprising an altered amino acid at position 631.